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NEPHROTOXIC EFFECTS OF MONOMETHYLHYDRAZINE IN MONKEYS

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The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 89–544, "Laboratory Animal Welfare Act," August 24, 1967.

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FOREWORD

This study was performed in support of research Project 7163, "Research on Biomechanisms and Metabolism." The work was performed from November 1966 to January 1968 in the Toxicology Branch, Toxic Hazards Division, Biomedical Laboratory, and at Mt. Sinai Hospital, New York, under Air Force Contract F33615-68-C-1284. The assistance rendered by Captain Gale Taylor, MSgt Joseph Young and TSgt Edgar Hagan is gratefully acknowledged.

The results of this study were presented at the Society of Toxicology Meeting on 6 March 1968 in Washington, D. C.

This technical report has been reviewed and is approved.

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ABSTRACT

The increased emphasis on use of monomethylhydrazine (MMH) as a propellant in space systems has made it necessary to determine the toxic effects of this compound. Previous studies indicated a nephrotoxic effect in dogs after MMH exposure. This study was designed to determine possible effects on kidney function in monkeys following single and repeated injections of MMH. Renal function tests and needle biopsies for electron microscopic examination of kidney tissue were performed on monkeys exposed to MMH. The left kidney of each animal was surgically translocated to a subcutaneous pocket and baseline needle biopsy samples taken; baseline renal function tests, i.e., glomerular filtration rate, renal plasma flow, and maximum tubular excretion rate, were determined. One group of monkeys was exposed to a single injection of 7.5 mg/kg MMH, one group to 2.5 mg/kg daily for 14 days, one group to 5.0 mg/kg every other day for 14 days, and one group to 5.0 mg/kg daily for 5-10 days. The renal function tests were repeated 24 hours after the final exposure and renal biopsy samples taken 48 hours after exposure. There was no statistically significant change in the renal function tests in any group. However, examination of the renal biopsy samples revealed major changes in the subcellular morphology in all groups of monkeys following MMH exposure.

INTRODUCTION

The increased emphasis on the use of monomethylhydrazine (MMH) as a propellant in space systems has prompted an intensive investigation into the toxic effects of this compound. Several investigators have described the toxicity (Weir, 1964), metabolism (Dost, 1966; Pinkerton, 1967), pathology (Back, 1966; Sopher, 1967), and effect on performance (Reynolds, 1966) of MMH in various experimental animals. Studies at the Aerospace Medical Research Laboratories at Wright-Patterson Air Force Base have shown that one of the major toxic effects of MMH in dogs is the production of severe renal damage. After exposure to subconvulsive levels of MMH, dogs exhibited hematuria, hemoglobinuria, methemoglobinuria, and cast formation. Histopathological examination of the kidneys revealed proteinaceous precipitates in the proximal tubules with occasional hemoglobin casts, moderate to severe degeneration of the proximal tubules with actual tubular necrosis present in many cases (Sopher 1967). Van Stee (1965) reported a decrease in tubular excretion in dogs following MMH exposure; there was also a decrease in glomerular filtration rate, which he attributed to a decrease in the renal plasma flow rate. The work described in this paper was performed to determine if the kidney malfunction and pathology observed in dogs after exposure to MMH could also be produced in monkeys following single and repeated exposures to MMH.

METHODS

Twenty monkeys, macaca mulatta, were selected, ten males and ten females, ranging in weight from 3 to 6 kilograms. In order to evaluate the morphologic and functional changes without killing the animals, tissue for electron microscopic examination was obtained by needle biopsy of the left kidney. To facilitate the biopsy procedure, the left kidney of each monkey was surgically translocated to a subcutaneous pocket (McCulley, 1964; Kaplan, 1967; Yunis, 1967). Eight weeks after the translocation surgery, baseline renal function tests were performed. Glomerular filtration rate (GFR) was determined by endogenous creatinine clearance, renal plasma flow (RPF) by sodium para amino hippurate clearance (PAH), and proximal tubular excretion (Tmax PAH) by total maximum PAH clearance (Kaplan, 1967). The animals were fasted overnight before the renal function tests and were allowed water ad libitum. The monkeys were anesthetized with intravenous (iv) sodium pentobarbital, approximately 30 mg/kg. An indwelling urinary catheter was placed in the bladder for urine collection. A 17-gauge iv catheter was inserted in the saphenous vein for infusions and a Cournand needle in the femoral artery for blood collection. The monkeys were hydrated with physiological saline for 45 minutes before the infusion of PAH to insure adequate urine flow. An initial loading dose of PAH, 12 mg/kg,

and a sustaining infusion of PAH, 0.3 mg/kg,minute, provided a plasma PAH level of 1-2 mg/100 ml for the determination of renal plasma flow. A loading dose of PAH, 300 mg/kg, and a sustaining infusion of PAH, 4.0 mg/kg/minute, providing plasma PAH levels of 20-40 mg/100 ml, were given to determine Tmax PAH. Creatinine clearance was measured simultaneously with RPF and Tmax PAH. There were three sampling periods of 15 minutes each for RPF and for Tmax PAH with an equilibration period of 30 minutes after each initial loading dose to provide adequate time for PAH blood levels to plateau. The results are reported on a body weight basis rather than surface area.

Twenty-four hours after the baseline renal function tests, a needle biopsy of the left kidney was performed under local anesthesia to obtain tissue for electron microscopy.

Six weeks after the baseline values were obtained, the monkeys were divided into five groups. Group I, the control animals, were injected with physiological saline and the other four groups were injected with MMH as shown in table I.

TABLE I
DOSAGE SCHEDULE

Group I Saline (4)	14 days
Group II MMH (4)	7.5 mg/kg single injection
Group III MMH (4)	2.5 mg/kg daily, 14 days
Group IV MMH (4)	5.0 mg/kg every other day, 14 days
Group V MMH (4)	5.0 mg/kg daily, 5-10 days
	1 monkey, 5 days
	1 monkey, 7 days
	2 monkeys, 10 days

The MMH was obtained from The Olin Matheson Co. and was diluted with water to an appropriate concentration so that the amount injected was between 0.4 and 0.8 ml. All injections were given intraperitoneally. Renal function tests were performed 24 hours after the final injection of MMH. MMH levels in the urine and blood were determined at this time (Reynolds, 1965). Forty-eight hours after the final injection of MMH, tissue was taken by needle biopsy for electron microscopy.

RESULTS

All monkeys except the controls had a loss of appetite during the course of exposure with a resultant weight loss. The animals in groups II and III had no other overt symptoms and appeared clinically well. Two of the four monkeys in group IV displayed emesis following the third injection. All of the monkeys in group V had emesis after the third injection, which continued intermittently throughout the course of exposure. Two of the four convulsed on the fourth and sixth days of exposure, and one of these monkeys exhibited hematuria and hemoglobinuria on the ninth day of exposure. All of the monkeys in group V were weak and lethargic and appeared clinically ill.

There were no detectable amounts of MMH in the blood or urine of any of the monkeys 24 hours after the final injection.

The average baseline and postexposure values for glomerular filtration rate, renal plasma flow, and tubular excretion for each experimental group are shown in table II. The results of these renal function tests indicated there was no statistically significant difference between baseline and post-MMH exposure values or between the control group and animals exposed to MMH.

TABLE II
GROUP AVERAGES

Group	I	II	III	IV	V
	Glomerular Filtration Rate ml/min,kg				
Baseline	3.41	2.92	3.77	3.37	3.59
Postexposure	3.61	3.15	3.21	3.76	2.92
	Renal Plasma Flow ml/min,kg				
Baseline	19.67	18.15	21.33	23.28	17.94
Postexposure	19.16	21.86	23.63	23.44	13.23
	Tubular Excretion mg/min,kg				
Baseline	2.70	2.71	2.59	3.06	2.81
Postexposure	2.93	2.79	2.50	2.76	2.51

The values for the individual monkeys in groups II, III, and IV showed some variation between pre-and postexposure either plus or minus. However, all of the monkeys in group V had a decrease in all three function tests, although the decrease was not statistically significant.

The electron micrographs of the tissue from the kidney biopsies indicated there were definite changes in the morphology of both proximal and distal tubule cells after MMH exposure. These changes were not uniformly distributed nor of uniform severity, but were observed in all tissue samples from MMH-exposed monkeys. The electron micrographs of the baseline and the control tissue specimens showed essentially normal cellular structure. The proximal tubule cells showed a moderate amount of apical vacuolization, normal brush border, normal nuclei, intact mitochondria, and the usual cellular interdigitations (fig. 1). Other cell organelles, such as the Golgi zone and microbodies were found in their usual distribution (fig. 2). The effects of MMH on cellular morphology were most pronounced in the monkeys in group II. The proximal and distal tubules were involved to approximately the same degree. The morphologic changes consisted primarily of cellular vacuolization, mitochondrial swelling with a loss of density in the mitochondrial matrix and partial disappearance of cristae. Some tubules showed only moderate changes with preservation of the cellular architecture (fig. 3). There was vacuolization in the basal portion of the cell as well as in the apical portion; the mitochondria showed mild swelling with only a slight decrease in the density. In more severely affected areas, these changes were much more pronounced (fig. 4). In some tubules the degree of mitochondrial swelling resulted in a marked decrease in the density of the mitochondrial matrix with a partial loss of cristae (fig. 5). In the most severely affected areas, the tubular cells were completely filled with vacuoles and the mitochondria could be recognized only by virtue of their double membranes and remnants of cristae (fig. 6). The morphologic changes in monkeys given multiple injections of MMH (groups III, IV, and V) were similar in nature, but were less severe than those seen in monkeys given a single injection (group II). The distal tubules were only minimally affected showing some diffuse vacuolization with occasional casts in the lumen (fig. 7). Some proximal tubules, however, showed such extreme vacuolization that the cellular architecture was completely disrupted (fig. 8). Other proximal tubules had only moderate vacuolization but had pronounced mitochondrial swelling with a marked decrease in the density of the mitochondrial matrix and a loss of cristae (fig. 9).

DISCUSSION

The changes in the morphology of the renal tubule cells after exposure to MMH were not reflected by any significant changes in renal function. Since tubular excretion is an energy-requiring process and the enzymes involved in energy production are concentrated in the mitochondria, it seems unlikely that the cells exhibiting the most severe damage would have adequate energy production required for maximal tubular excretion. Since the morphologic changes observed in the cells varied in the degree of severity and in distribution, it seems probable that there were intact and unaffected nephrons in sufficient numbers to maintain normal function. The nephrotoxic effects of exposure to MMH are much more severe in dogs than in monkeys. This may be due partially to the severe hemoglobinuria and methemoglobinuria observed in dogs, resulting in a hemoglobinuric nephropathy. However, in addition, there does appear to be a direct toxic effect on the tubular epithelial cells resulting in a decreased

tubular excretion (Van Stee, 1965; Sopher, 1967). When given similar doses of MMH with comparable levels of MMH in the blood, monkeys do not exhibit a similar nephrotoxic response. There are definite cellular morphologic changes, but certainly not of a comparable severity. The reasons for this difference in response between dogs and monkeys are unclear at this time.

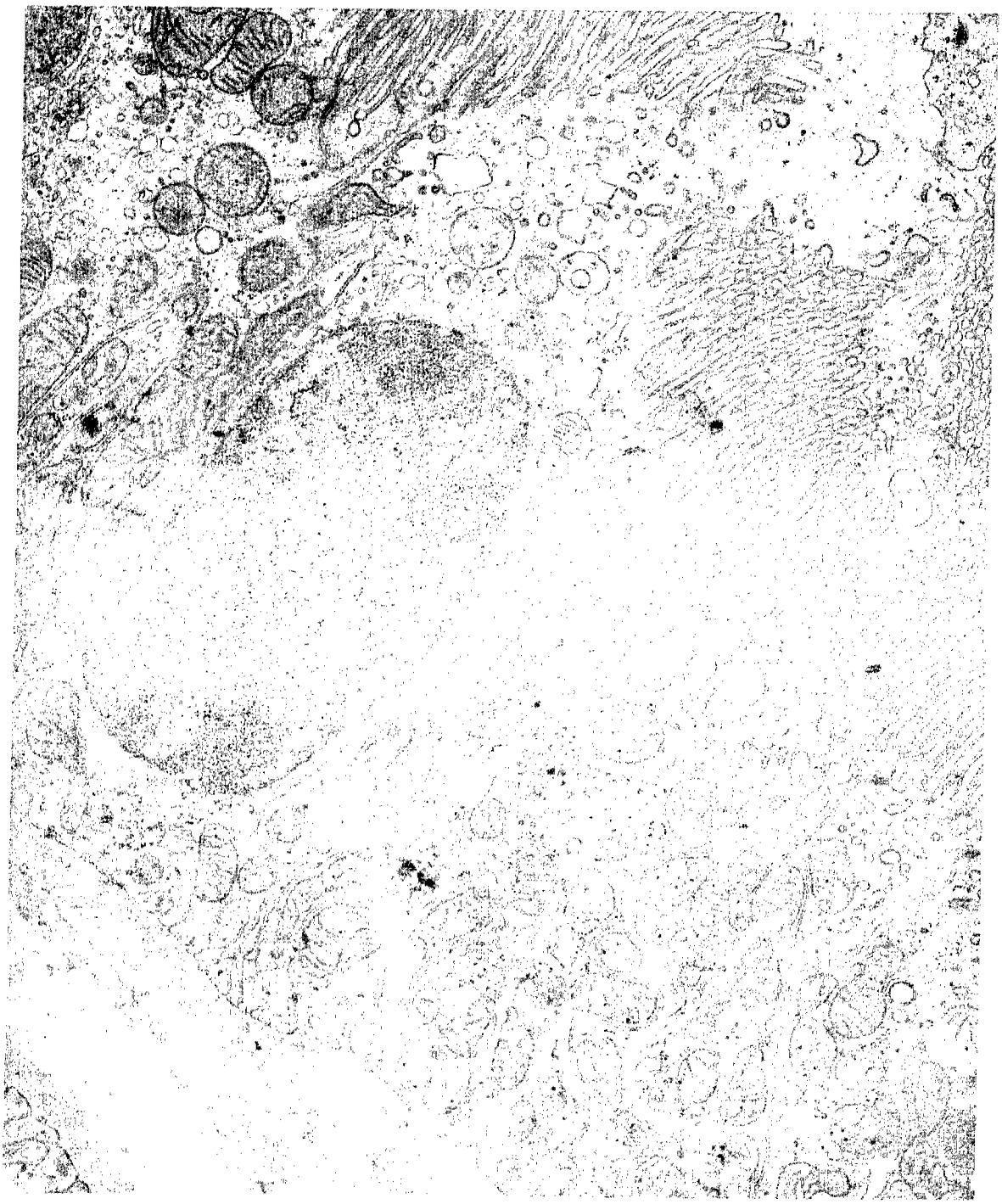

Fig. 1 Normal monkey, proximal tubule. Brush border at upper right, basement membrane at lower left. Mitochondria show normal internal architecture. Below brush border, normal apical vacuolization. Electron micrograph, X 15,405

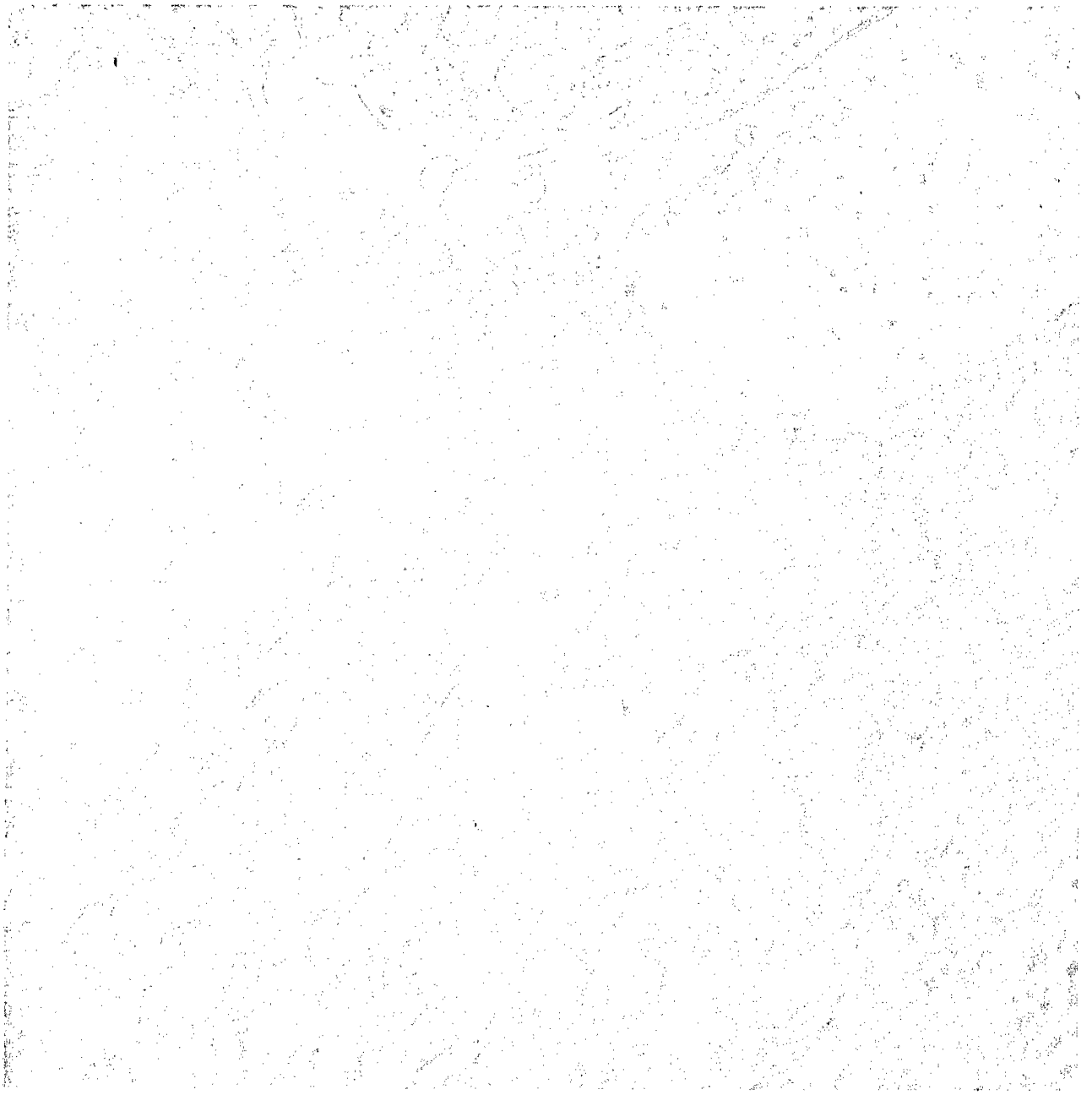

Fig. 2 Normal monkey, proximal tubule. Top: lumen partially filled with cytoplasmic "blebs"; Center: brush border. Bottom: apical portion of proximal tubular cells showing normal mitochondria, golgi zones, and microbodies. Electron micrograph, X 20,800

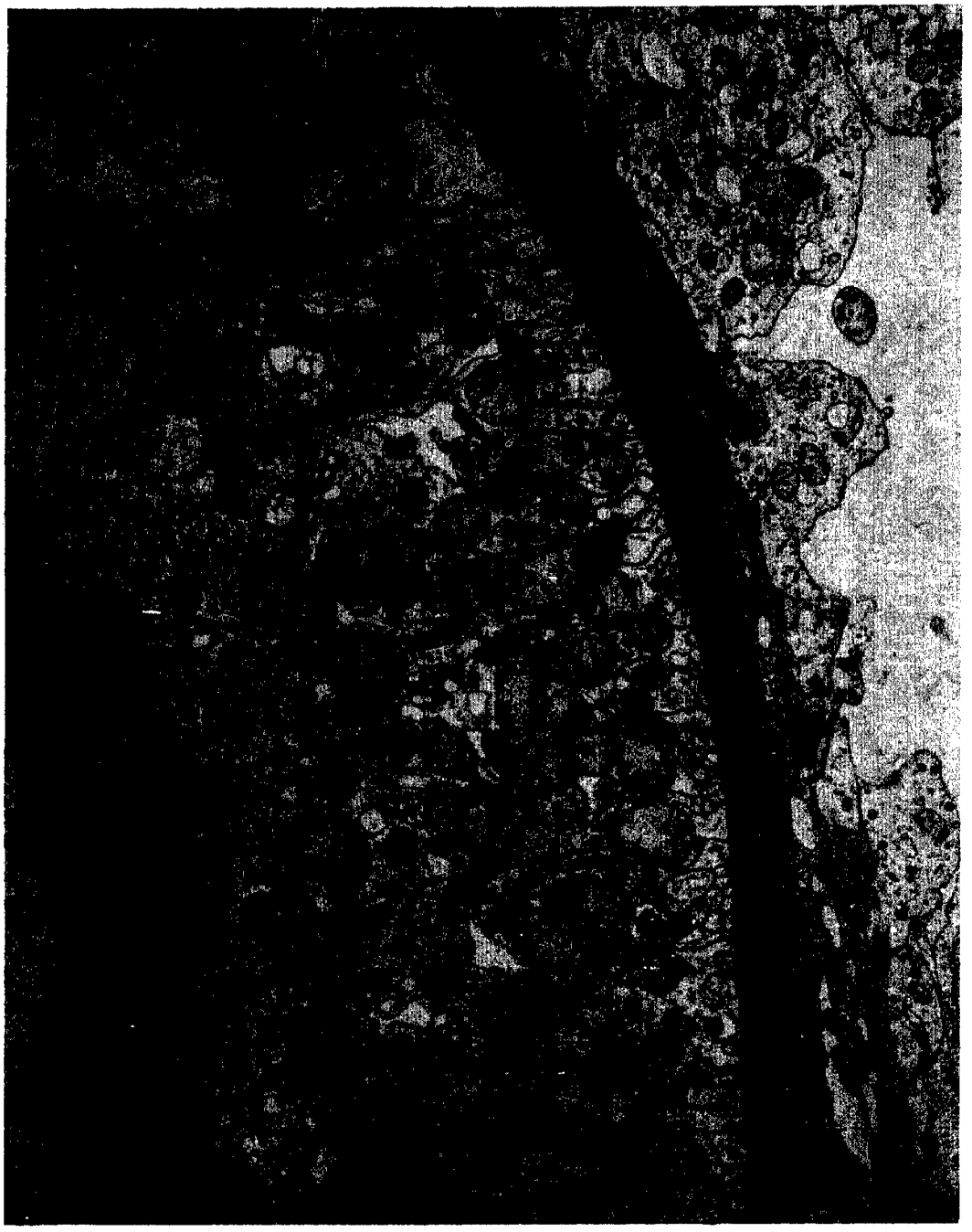

Fig. 3 Monkey, single injection of MMH, proximal tubule. Tubular lumen at left, capillary lumen at right. Moderate swelling of mitochondria and moderate diffuse vacuolization.

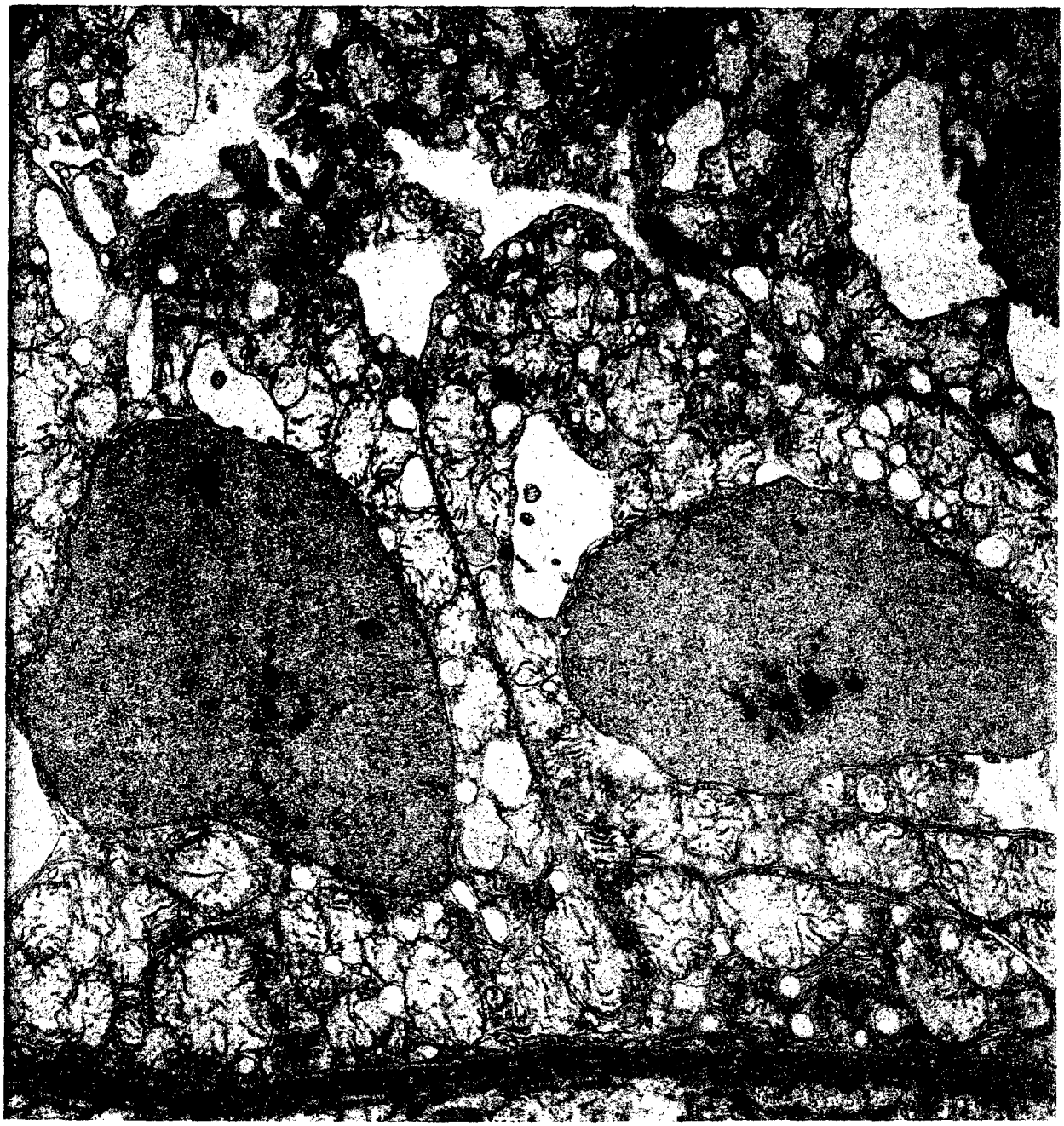

Fig. 4 Monkey, single injection of MMH, distal tubule. Lumen near top, basement membrane near bottom. Mitochondrial swelling and diffuse vacuolization, both moderately severe. Electron micrograph, X 13, 593

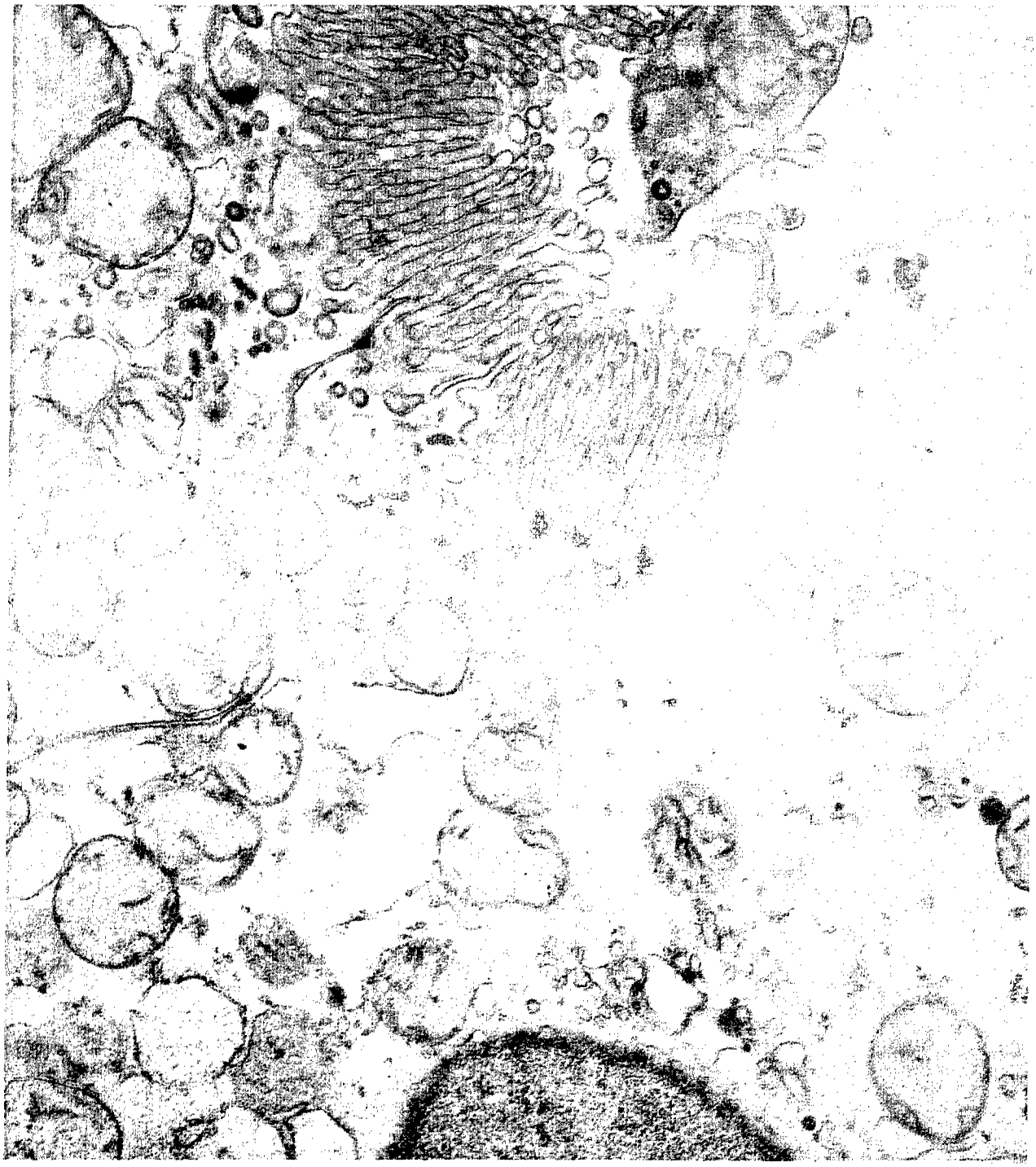

Fig. 5 Monkey, single injection of MMH, proximal tubule. Lumen at upper right. Mitochondria are increased in size, their matrix is decreased in density, and there is a loss of cristae. Electron micrograph, X 23,587

Fig. 6 Monkey, single injection of MMH, distal tubule. Lumen at left. Diffuse vacuolization and mitochondrial swelling, both severe. Electron micrograph, X 23,587

Fig. 7 Monkey, multiple injections of MMH, distal tubule. Slight vacuolization with essentially normal mitochondria. Electron micrograph, X 23,587

Fig. 8 Monkey, multiple injections of MMH, Proximal tubule. Distortion of architecture due to large vacuoles and severe mitochondrial swelling. Electron micrograph, X 13,593

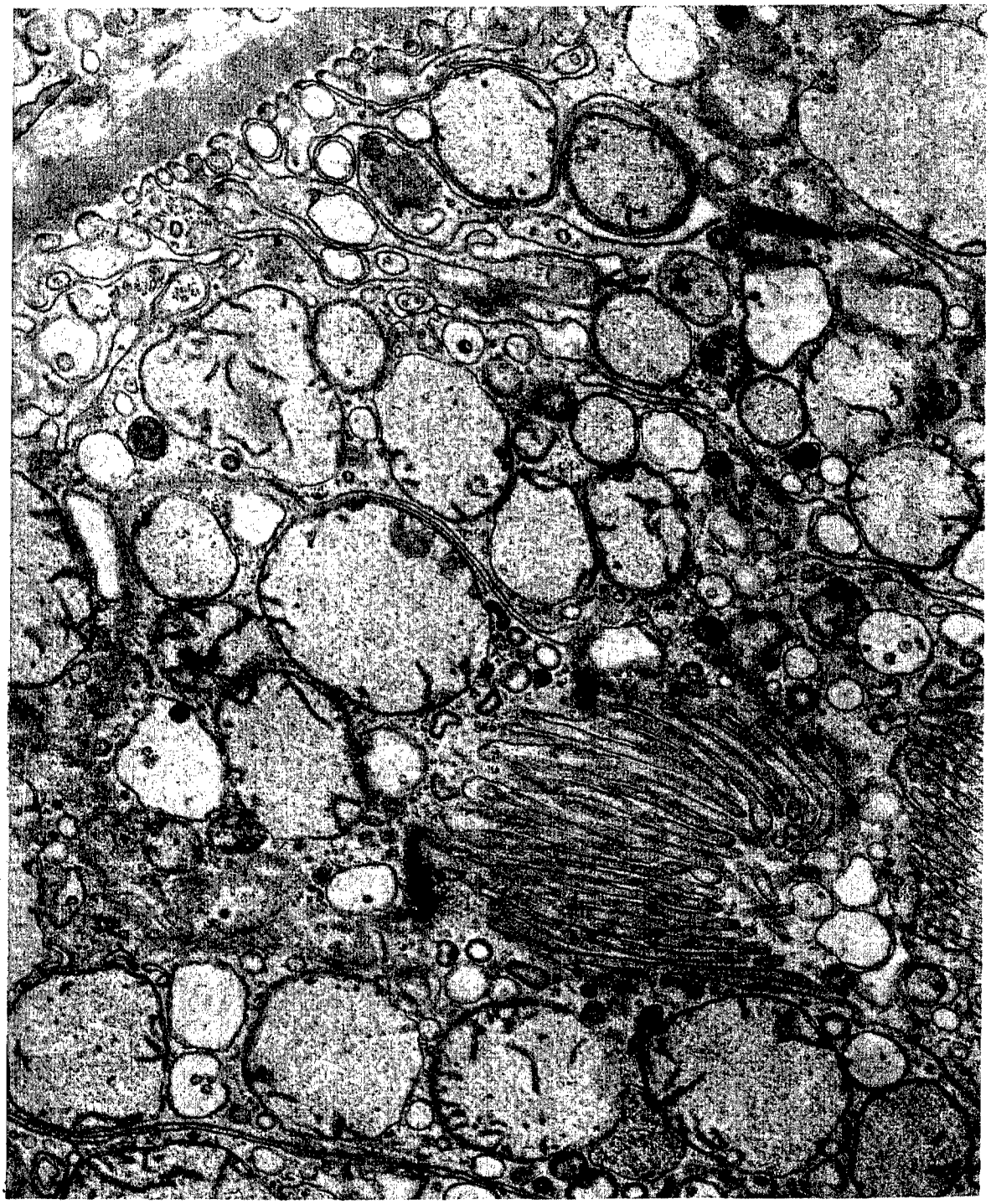

Fig. 9 Monkey, multiple injections of MMH, Proximal tubule. Extreme mitochondrial swelling. Electron micrograph, X 23,587

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13. ABSTRACT

The increased emphasis on use of monomethylhydrazine (MMH) as a propellant in space systems has made it necessary to determine the toxic effects of this compound. Previous studies indicated a nephrotoxic effect in dogs after MMH exposure. This study was designed to determine possible effects on kidney function in monkeys following single and repeated injections of MMH. Renal function tests and needle biopsies for electron microscopic examination of kidney tissue were performed on monkeys exposed to MMH. The left kidney of each animal was surgically translocated to a subcutaneous pocket and baseline needle biopsy samples taken; baseline renal function tests, i. e., glomerular filtration rate, renal plasma flow, and maximum tubular excretion rate, were determined. One group of monkeys was exposed to a single injection of 7.5 mg/kg MMH, one group to 2.5 mg/kg daily for 14 days, one group to 5.0 mg/kg every other day for 14 days, and one group to 5.0 mg/kg daily for 5-10 days. The renal function tests were repeated 24 hours after the final exposure and renal biopsy samples taken 48 hours after exposure. There was no statistically significant change in the renal function tests in any group. However, examination of the renal biopsy samples revealed major changes in the subcellular morphology in all groups of monkeys following MMH exposure.

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